ZooKeys 675: 1–25 (2017)
doi: 10.3897/zookeys.675.12024
http://zookeys.pensoft.net

RESEARCH ARTICLE

A peer-reviewed open-access journal
ZooKeys
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A new species of *Rhodnius* from Brazil (Hemiptera, Reduviidae, Triatominae)

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Academic editor: *G. Zhang* | Received 31 January 2017 | Accepted 30 March 2017 | Published 18 May 2017

http://zoobank.org/73FB6D53-47AC-4FF7-A345-3C19BFF86868

Citation: Rosa JA, Justino HHG, Nascimento JD, Mendonça VJ, Rocha CS, Carvalho DB, Falcone R, Azeredo-Oliveira MTV, Alevi KCC, Oliveira J (2017) A new species of *Rhodnius* from Brazil (Hemiptera, Reduviidae, Triatominae). ZooKeys 675: 1–25. https://doi.org/10.3897/zookeys.675.12024

Abstract

A colony was formed from eggs of a *Rhodnius* sp. female collected in Taquarussu, Mato Grosso do Sul, Brazil, and its specimens were used to describe *R. taquarussuensis* **sp. n.** This species is similar to *R. neglectus*, but distinct characters were observed on the head, thorax, abdomen, female external genitalia and male genitalia. Chromosomal differences between the two species were also established.

Keywords

Brazil, cytotaxonomy, new species, *Rhodnius*, taxonomy, Triatominae

Introduction

In the subfamily Triatominae, the genera *Panstrongylus* (15 species), *Triatoma* (74 species) and *Rhodnius* (20 species) are of particular epidemiological importance, although the other 15 genera (containing 43 species) can also transmit *Trypanosoma cruzi*, which

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is the etiological agent of Chagas disease (Poinar 2013, Galvão 2014, Mendonça et al. 2016, Souza et al. 2016). Among the 152 species within the subfamily there are two fossils: *T. dominicana* Poinar, 2005 and *P. hispaniolae* Poinar, 2013.

The first two species identified as belonging to the genus *Rhodnius* were described by Stal (1859): *R. nasutus* and *R. prolixus*. From that year until 1979, a total of 12 species were identified (Lent and Wygodzinsky 1979). In 2003, Galvão et al. considered 16 valid species. The 17th, 18th, 19th and 20th species in that genus were respectively *R. zeledoni* Jurberg et al. 2009; *R. montenegrensis* Rosa et al. 2012; *R. barretti* Abad-Franch et al. 2013, and *R. marabaensis* Souza et al. 2016.

Most *Rhodnius* species live in palm trees, and several cases of transmission of Chagas disease have been associated with the consumption of açaí containing feces of triatomines infected by *T. cruzi* (Ferreira, Branquinho & Leite, 2014; Ministério da Saúde, Brasil, 2017). Apart from such cases, which occur more frequently in the northern region of the country, it is worth noting that *R. neglectus* was found in palm trees (species of *Roystonea*, *Syagrus* and *Acrocomia*] in the city of Araçatuba, São Paulo, in 2009, as well as in palm trees (*Livistona australis*) located in the central square of the city of Monte Alto, São Paulo, in February 2012 (Rodrigues et al. 2014, Carvalho et al. 2013, respectively).

Based on morphological, morphometric and cytogenetic characters, this paper describes *R. taquarussuensis* sp. n., which is similar to *R. neglectus*. The first collected specimen of *R. taquarussuensis* was a female that invaded a domicile and laid eight eggs. The colony formed from those eggs resulted in the specimens used in this description.

Materials and methods

Morphological identification and description

On 10 November 2010 a female of *Rhodnius* sp. invaded a rural dwelling (22°29'07.7"S; 53°21'08.9'W) in the city of Taquarussu, Mato Grosso do Sul, Brazil, and was captured (Fig. 1). That specimen remained alive for a few days and laid eight eggs (Fig. 2). By means of macroscopic identification and subsequent optical microscopy (OM) and using the key of Lent and Wygodzinsky (1979), a clear similarity with *R. neglectus* was noticed. In view of that, all characters observed and documented for *Rhodnius* sp. were checked for *R. neglectus* CTA 229, which is a colony that has been kept since June 27, 2011 at the Triatominae Insectarium of the Faculty of Pharmaceutical Sciences, UNESP, Araraquara (FCFAR/UNESP). The temperature, humidity and light cycle conditions are not controlled due to the insect's biodiversity, but these parameters are measured daily, varying the temperature between 20-35ºC and humidity 50-80%. Insects kept in colonies are fed directly on ducks every 15 days and consists of specimens from the Brazilian National and International Triatominae Taxonomy Reference Laboratory at the Oswaldo Cruz Institute in Rio de Janeiro, Brazil (LNIRTT). The colony was kindly provided by Dr. José Jurberg and Dr. Cleber Galvão, and the specimens that originated it were collected in northern Formoso, Goiás, Brazil.

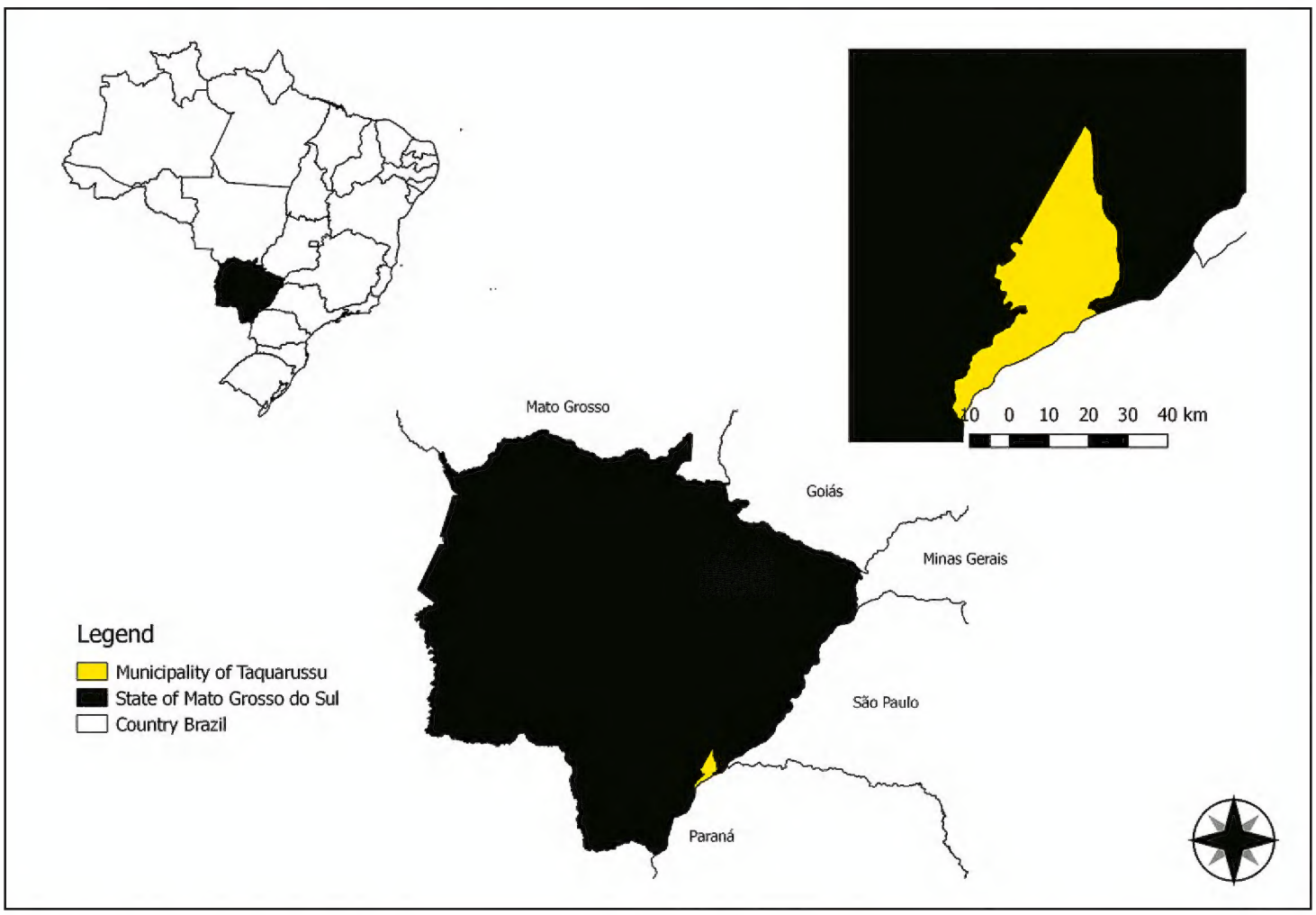

Figure 1. Localization of Taquarussu - MS where female of *R. taquarussuensis* sp. n. is collected (22°29'233"S, 053°21'107"W).

A colony was formed from the eight eggs laid by the *R. taquarussuensis* sp. n. female and identified as Araraquara Triatominae Colony (CTA) 277. The specimens of that colony were used to describe *R. taquarussuensis* sp. n.

Morphological study

The morphological study by OM and scanning electron microscopy (SEM) consisted of the observation of the head, thorax and abdomen of 30 adult females and 30 adult males, as well as 40 eggs of *R. taquarussuensis* sp. n. and the same number of specimens of *R. neglectus*, according to Barata (1981), Quintero (2003), Obara et al. (2007), Rosa et al. (2012), Rosa et al. (2014), Souza et al. (2016) (Figs 3–9).

Female external genitalia were observed from the dorsal, posterior, and ventral sides (Fig. 6) by SEM, according to Rosa et al. (2010). The study of the male genitalia was carried out by OM (Figs 8, 9), following a technique developed by Jader de Oliveira based on Gallati (2016). The denominations used were those defined by Lent and Jurberg (1969).

The Leica MZ APO stereoscope from the Faculty of Pharmaceutical Sciences, UNESP, Araraquara, and the scanning electron microscope Topcon SM-300 located in the Department of Physical Chemistry at the Chemistry Institute, UNESP, Araraquara, were used for observation and capture of images.

Table 1. Mean of measurement (mm) of 15 females and 15 males of *R. taquarussuensis* sp. n. and *R. neglectus*.

	Female		Male	
	R. taquarussuensis	***R.neglectus***	***R. taquarussuensis***	***R. neglectus***
TL	17,25	17,25	**15,24**	**15,96**
MLA	9,86	10,03	**8,41**	**9,05**
MLT	**5,00**	**4,11**	**4,54**	**3,79**
R1	**0,75**	**0,92**	**0,71**	**0,92**
R2	**3,38**	**3,58**	**3,16**	**3,59**
R3	**0,85**	**0,95**	**0,77**	**0,96**
HL	**4.44**	**5,81**	**4.20**	**5,24**
EO	**1.53**	**1,98**	**1.42**	**1,80**
IE	**0.61**	**0,77**	**0.54**	**0,66**
PO	**1.00**	**3,67**	**1.04**	**3,37**
AO	**2.62**	**0,90**	**2.44**	**0,91**
AT	**1.93**	**2,30**	**1.82**	**2,30**
SC	1,96	2,03	1,69	1,86
A1	0,45	0,55	0,29	0,59
A2	3,56	4,13	2,24	4,35
A3	2,03	2,43	1,28	2,50
A4	1,36	1,82	0,87	1,92
Eggs	***R. taquarussuensis***		***R. neglectus***	
TE	**1,72**		**1,62**	
OO	**0,49**		**0,52**	

*30 eggshells were used for each species.

TL, Total length of the triatomine; **MLA**, maximum length of the abdomen; **MLT**, maximum length of the thorax; **R1**, **R2** and **R3**, lengths of first, second, and third rostral segments, respectively; **HL**, head length; **EO**, external distance between ocelli; **IE**, inner distance between eyes; **PO**, postocular distance (excluding neck); **AO**, anteocular distance; **AT**, antenniferous tubercle; **SC**, Scutellum; **A1**, **A2**, **A3** and **A4**, 1st, 2nd, 3rd, and 4th left antennal segments, respectively; **TE**, Total egg length; **OO,** egg opercular opening. The values in bold were significant at $\alpha = 0.05$, using unpaired t-test.

Morphometric study

In the morphometric study by OM, 15 egg shells, 15 females and 15 males from the colony were measured, the same being done for *R. neglectus* CTA 229 (Table 1).The parameters measured were: total length, width of thorax and abdomen, length of the scutellum, three segments of the proboscis and four segments of the antenna, as well as five parameters of the head following Dujardin et al. (1999). Eggs had their length and the diameter of the opercular opening measured. The wings of *R. taquarussuensis* sp. n. and *R. neglectus* were studied by geometric morphometry using seven anatomical landmarks, according to parameters established by Gurgel-Gonçalves et al. (2008), as well as based on Rosa et al. (2012).

The observations and measurements were carried out on a Leica MZ APO stereoscope and the Motic Images Advanced System version 3.2.

Cytogenetic identification

In this study ten male specimens of *R. taquarussuensis* sp. n. were used for C and CMA_3/DAPI–banding analyses and ten male specimens of *R. neglectus* were used for CMA_3/DAPI–banding analyses. After being lacerated and placed on the slide, the seminiferous tubules underwent cytogenetic procedures following the C-banding (Sumner 1972) and CMA_3/DAPI-banding protocols [Schimid 1980, with modifications according to Severi-Aguiar et al. 2006]. C-banding was analyzed under a Jenaval (Zeiss) MO connected to a digital camera and the Axio Vision LE 4.8 image analyzer (Copyright ©2006-2009 Carl Zeiss Imaging Solutions Gmb H), whereas CMA_3/DAPI-banding was analyzed using Zeiss-Axioskop and Olympus BX-FLA fluorescence microscopes (FM).

Taxonomy

Family Reduviidae Latreille, 1807
Subfamily Triatominae Jeannel, 1919
Genus *Rhodnius* Stål, 1859

***Rhodnius taquarussuensis* sp. n.**
http://zoobank.org/16C7EE86-3C36-4BA9-BDFC-E914CC4C2F80
Figure 2

Holotype. BRAZIL: Mato Grosso do Sul: Taquarussu; Residence, 22°29'07.7"S; 53°21'08.9'W, 10 November 2010 H. E. G. Justino. UNESP (♀).

Paratypes. BRAZIL: Colony formed from eggs obtained from the holotype: Araraquara: Triatominae Insectarium of the Faculty of Pharmaceutical Sciences, Araraquara, January 3, 2017, J. A. da Rosa, UNESP (25 ♂ 25 ♀).

Additional paratypes. CTIOC - Collection of Triatomines of the Oswaldo Cruz Institute, Rio de Janeiro - Brazil (2 ♂ 2 ♀). Entomological Reference Collection of the Faculty of Public Health - USP, São Paulo -Brazil (1 ♂ 1 ♀). Collection of the Institute of Entomology of the Metropolitan University of Education Sciences (IEUMCE), Santiago - Chile (2 ♂ 2 ♀).

Etymology. The name *Rhodnius taquarussuensis* sp. n. was chosen because this species was found in the city of Taquarussu, Mato Grosso do Sul, Brazil.

Diagnosis. *Rhodnius taquarussuensis* sp. n. is close to *R. neglectus*, their differences being the color and a variety of morphological, morphometric and cytogenetic characters (Tables 1, 2). The general color of *R. taquarussuensis* sp. n. is brown, whereas *R. neglectus* is dark brown, almost black. This difference is particularly noticeable on the hind wings. The stridulatory sulcus of *R. taquarussuensis* sp. n. is brown at the base and black on the sides, whereas on *R. neglectus* it is completely black.

On the head, differences were noticed on the vertex, genae, antennae and triangular furrow of the first segment of the rostrum. The vertex of the head of *R. taquarussuensis*

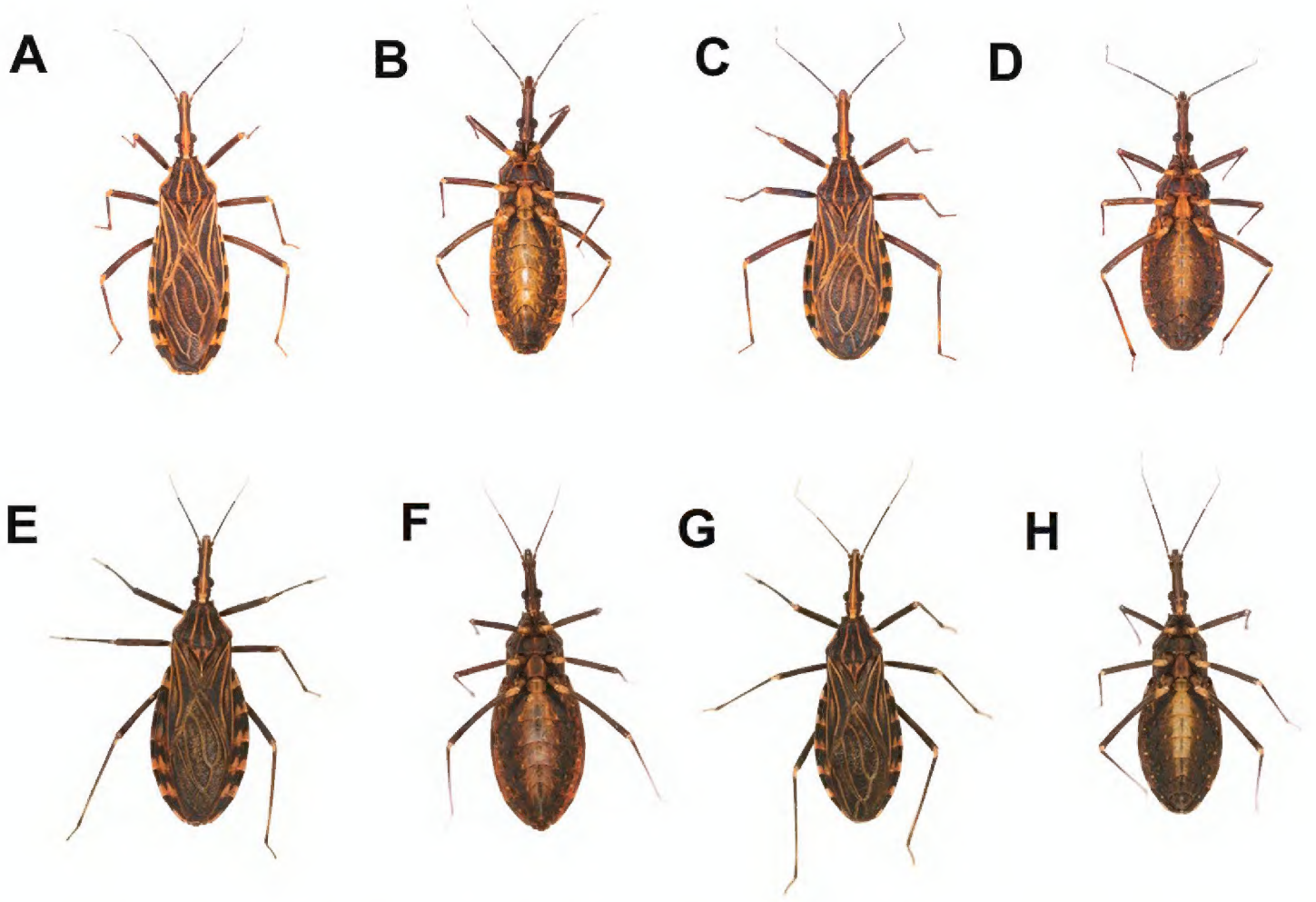

Figure 2. *R. taquarussuensis* sp. n. female **A** dorsal side **B** ventral side, *R. taquarussuensis* sp. n. male **C** dorsal side **D** ventral side, *R. neglectus* female **E** dorsal side **F** ventral side), *R. neglectus* male **G** dorsal side **H** ventral side.

sp. n. is quite visible, whereas on *R. neglectus* it is not (Fig. 3A, B, D, E). The genae of *R. taquarussuensis* sp. n. are longer than those of *R. neglectus* (Fig. 3A, D).On *R. taquarussuensis* sp. n. the 10th part of the second segment of the antenna is brown; on *R. neglectus*, though, only the basis has that color. The triangular furrow of the first segment of the rostrum, towards the second segment, ends in a filamentous way on *R. taquarussuensis* sp. n. and in a rounded way on *R. neglectus* (Fig. 3C, F). On the thorax, differences can be found on the pronotum, wings, scutellum, prosternum, mesosternum and metasternum (Figs 4, 5). The membranous portion of the hind wings is brown on *R. taquarussuensis* sp. n. and dark brown on *R. neglectus*. The scutellum ends in a rounded apex on *R. taquarussuensis* sp. n. and in a filamentous apex on *R. neglectus* (Fig. 4A, B). On *R. taquarussuensis* sp. n. the apex of the scutellum covers the final portion of the urotergite I process, while on *R. neglectus* the apex of the process of the urotergite I is perfectly visible (Fig. 4A, B). The lines limiting the stridulatory sulcus are straight on *R. taquarussuensis* sp. n. and narrowed in the anterior third on *R.neglectus* (Fig. 5A, B). On *R. taquarussuensis* sp. n. the basis of the stridulatory sulcus is brown and the sides are black, whereas on *R. neglectus* the entire stridulatory sulcus is black. The central region of the limit between the mesosternum and the metasternum is regular and half-moon shaped on *R. taquarussuensis* sp. n., while on *R. neglectus* it is pronounced and slightly irregular (Fig. 5C, D). The beginning of the metasternum is narrow on *R. taquarussuensis* sp. n.

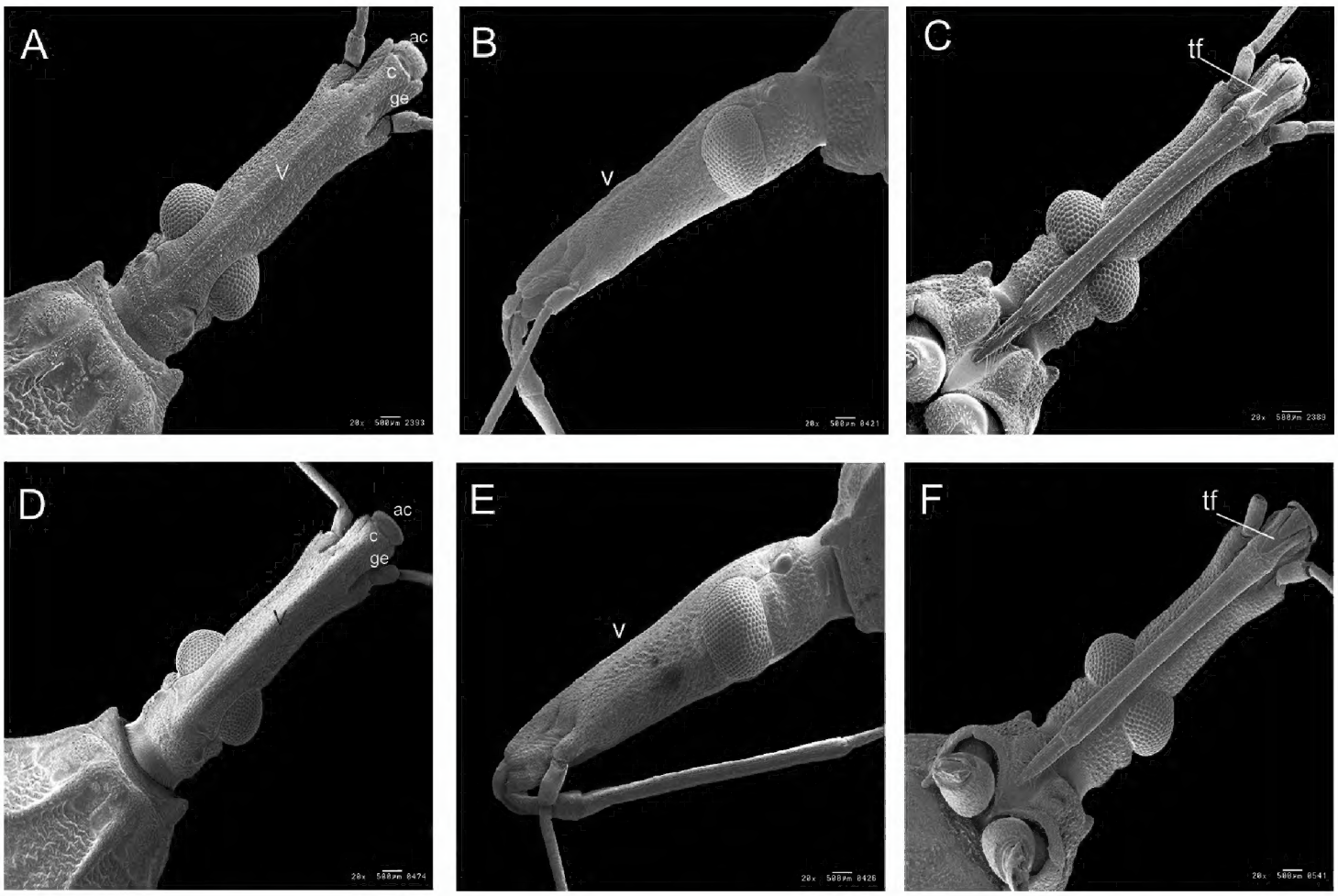

Figure 3. Head by SEM of *R. taquarussuensis* sp. n. **A** dorsal view **B** lateral view, **C** ventral view, *R. neglectus* **D** dorsal view **E** lateral view **F** ventral view. v: vertice, ge: gena, c: clypeus, ac: anteclypeus, tf: triangular furrow.

Table 2. Main distinguishing characters between *R.taquarussuensis* sp. n. and *R. neglectus.*

Distinguishing characters		**Species**	
		R.taquarussuensis	***R. neglectus***
Overall color		Brown	Dark brown
Genae		Lengthier longer	Longer
Vertex		Quite visible	Not visible
Ventral triangular furrow		Filamentous way	Rounded way
Scutellum		Covers the final portion of the urotergite I process	The apex of the process of the urotergite I is perfectly visible
Stridulatory sulcus		Straight	Waisted
Mesothorax		Half-moon shaped and regular	Pronounced and slightly irregular
Female external genitalia	Dorsal side	10th segment presents a concavity in the middle portion	10th segment is straight
	Posterior side	The limits of the 9th segment with gonocoxite VIII are curve	The limits of the 9th segment with gonocoxite VIII are straight
	Ventral side	There is a concavity in the external limit with the 10th segment	There is a straight line in the external limit with the 10th segment
Male genitalia	Phallothecal sclerite	Trapezoidal shape	Rounded shape
	Tip of parameres	Thinner	Thin
Heterochromatin in the autosomes		Present	Absent
CMA^{+} in autosomes		Present	Absent

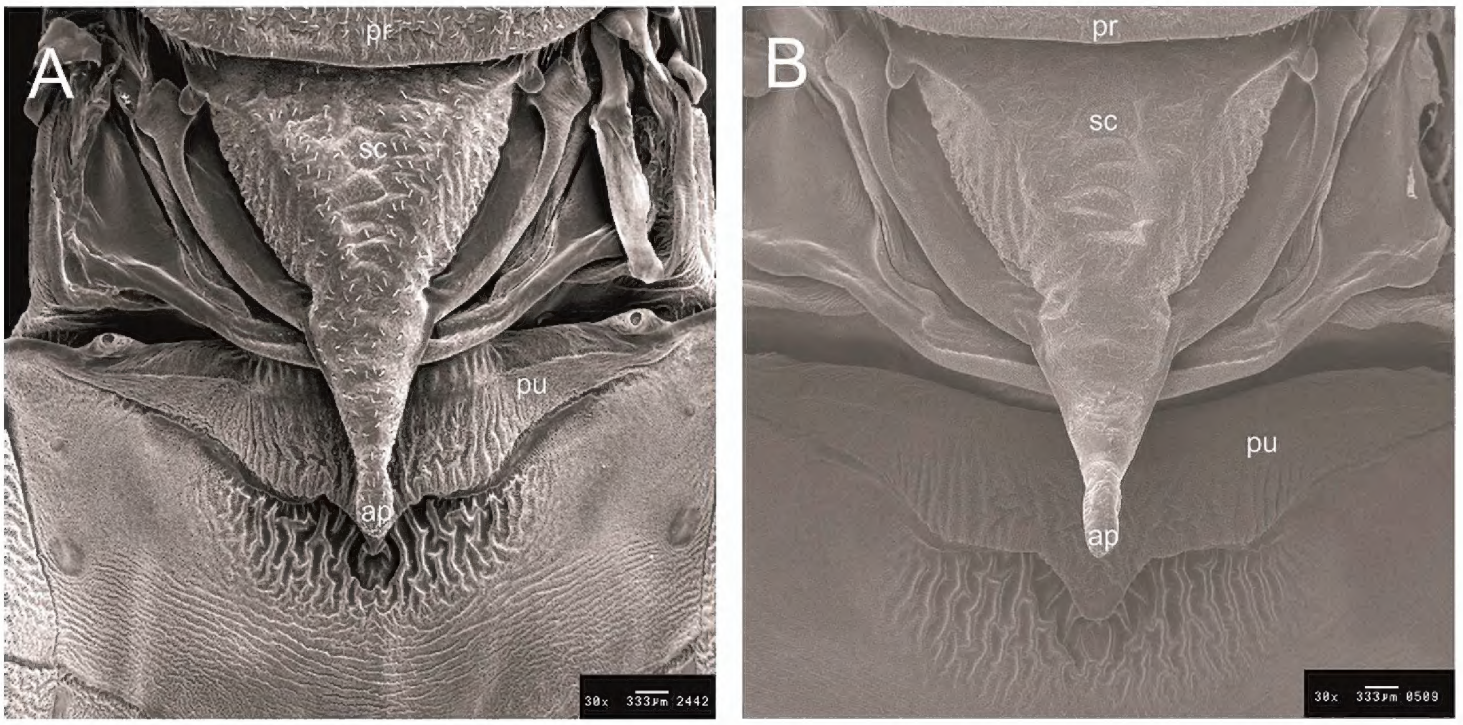

Figure 4. Escutellum and process of I urotergit by SEM. **A** *R. taquarussuensis* sp. n. **B** *R. neglectus*. pr: pronotum, sc: escutelum, pu: process of I urotergit, ap: apex of escutelum.

and wide on *R. neglectus* (Fig. 5C, D). The ventral abdomen of *R. taquarussuensis* sp. n. is light brown, and that of *R. neglectus* is dark brown (Fig. 2). The terminal portion of the paramere of the male genitalia of *R. taquarussuensis* sp. n. is thinner than that of *R. neglectus* (Fig. 9A, C). The dorsal phallothecal sclerite has a trapezoidal shape on *R. taquarussuensis* sp. n. and is rounded on *R. neglectus* (Fig. 8C, D). The external limit of the 10th segment of the dorsal side of the female external genitalia of *R. taquarussuensis* sp. n. presents a concavity in the middle portion, whereas on *R. neglectus* that limit is straight (Fig. 6A, B). From posterior view, the limits of the 9th segment with gonocoxite VIII are curve on *R. taquarussuensis* sp. n. and straight on *R. neglectus*, and the superior line limiting the 10th and 9th segments is straight on *R. taquarussuensis* sp. n. and curve on *R. neglectus* (Fig. 6C, D). In the ventral side of the female external genitalia of *R. taquarussuensis* sp. n. there is a concavity in the external limit with the 10th segment that is also noticed from dorsal view; on *R. neglectus* that limit is a straight line. From ventral view, the external limits of the 9th segment of the female external genitalia are curve on *R. taquarussuensis* sp. n. and straight on *R. neglectus* (Fig. 6E, F).

Among the 19 characters measured, 12 showed significant differences between *R. taquarussuensis* sp. n. and *R. neglectus* in both sexes and also the eggs of both species. Two characters showed differences only between males, and five characters did not show significant differences (Tables1, 2).

Description. A total of 15 adult females and 15 adult males of *R. taquarussuensis* sp. n. and *R. neglectus* were measured, as well as 30 eggs shells of both species. Such measurements are detailed in Table 1.

The head of *R. taquarussuensis* sp. n. has a prominent brown vertex contrasting with the black sides. The clypeus is well defined. The genae are large, visible and dark brown, moving towards the anteclypeus (Figs 2A, C, 3 A, B). The limits between the genae and the clypeus are brown.

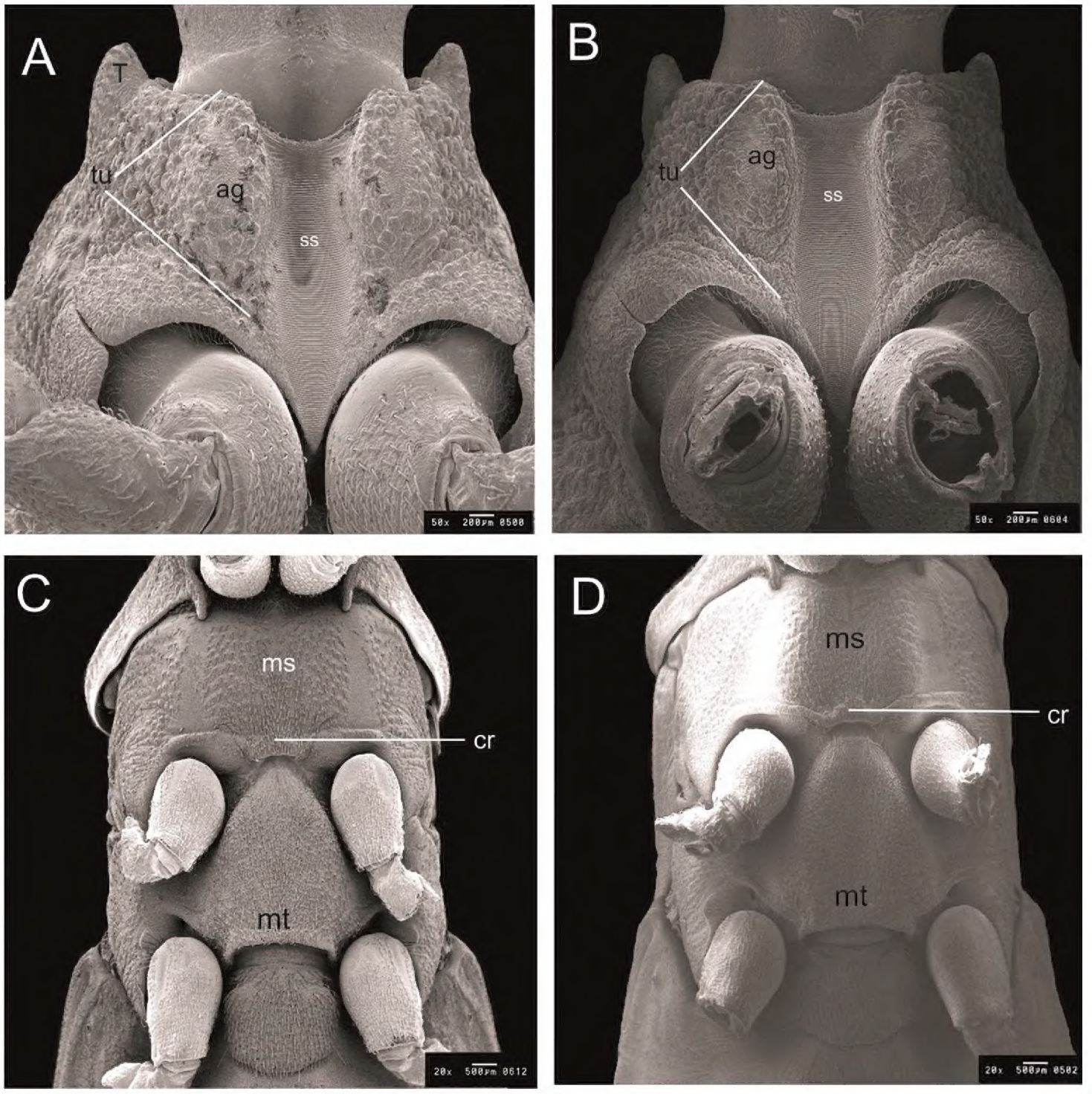

Figure 5. Thorax ventral by SEM. **A, C** *R. taquarussuensis* sp. n. **B, D** *R. neglectus*. ss: stridulatory sulcus, ms: mesosternum, mt: metasternum, tu: tubercle, ga: glabrous area, cr: central region.

The first segment of the antennae is black with mixes of brown. The articulation between the first and second segment of the antennae is brown. Roughly all the 10th part of the beginning of the second antennal segment is brown. The second segment is mostly black. In the articulation between the second and third antennal segment there is a black ring followed by a brown one. The beginning of the third segment (around 1/3) is black and the remaining portions (2/3) are brown. The articulation between the third and fourth antennal segment is brown. The beginning of the fourth segment is black and the remaining portions are brown with mixes of black (Fig. 2A, C).

The eyes are black and the ocelli are brown. The neck has a brown central dorsal strip flanked by two (1+1) black, narrower strips. The ventral portion of the neck between the ocelli is dark brown (Fig. 2A, B, C, D).

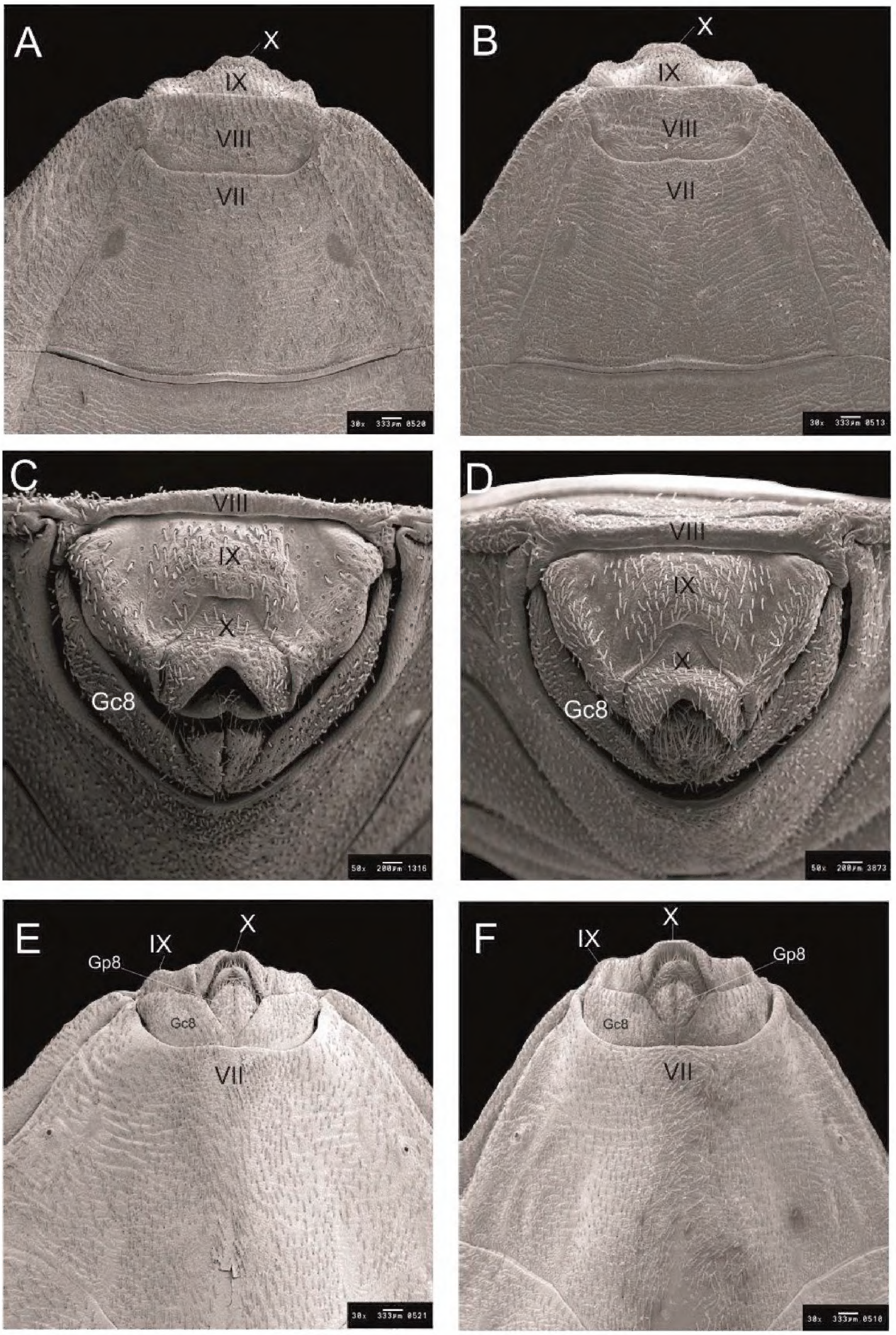

Figure 6. Female external genitalia by SEM *R. taquarussuensis* sp. n. **A** dorsal view, **C** posterior view **E** ventral view, *R. neglectus* **B** dorsal view **D** posterior view **F** ventral view. Gc 8: gonocoxite VIII; Gc 9: gonapophyse IX; Gp 8: gonapophyse VIII; VII, IX: tergites; X: segment.

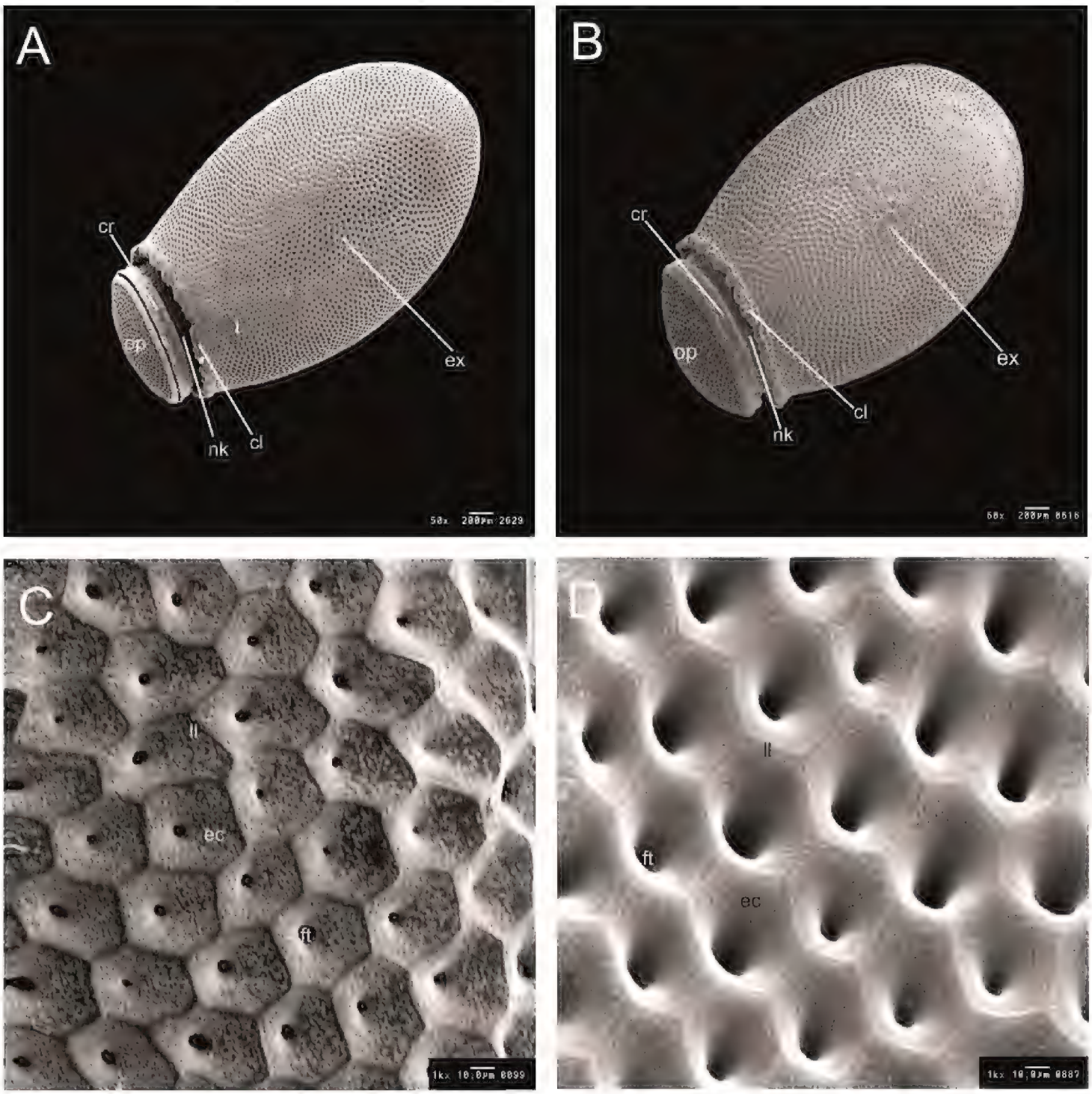

Figure 7. Eggs general vision and egg exochorium detail of *R. taquarussuensis* sp. n. (**A, C**), *R. neglectus* (**B, D**). cl: colar, cr: chorial rim, ex: exochorium, nk: neck, op: operculum, ec: exochorium cell, ft: follicular tubes, ll: limiting line.

The pronotum of the thorax of *R. taquarussuensis* sp. n. has a trapezoidal shape and is limited by a brown carina. In the antero posterior direction the pronotum has other two brown carina in the middle portion and six black strips. The three carina and the three brown strips are interspersed with the six black strips, which are larger. The collar (first portion of the pronotum) in the central part is brown and is followed by two (1+1) black glabrous areas and the two (1+1) antero lateral angles. The anterior portion of the pronotum consists of three anterior lobes which are clearly distinct from the posterior portion (hindlobe). Those three anterior lobes are limited by the carina and on each of them there are two black glabrous areas with a lengthy and irregular outline (Fig. 2A, C).

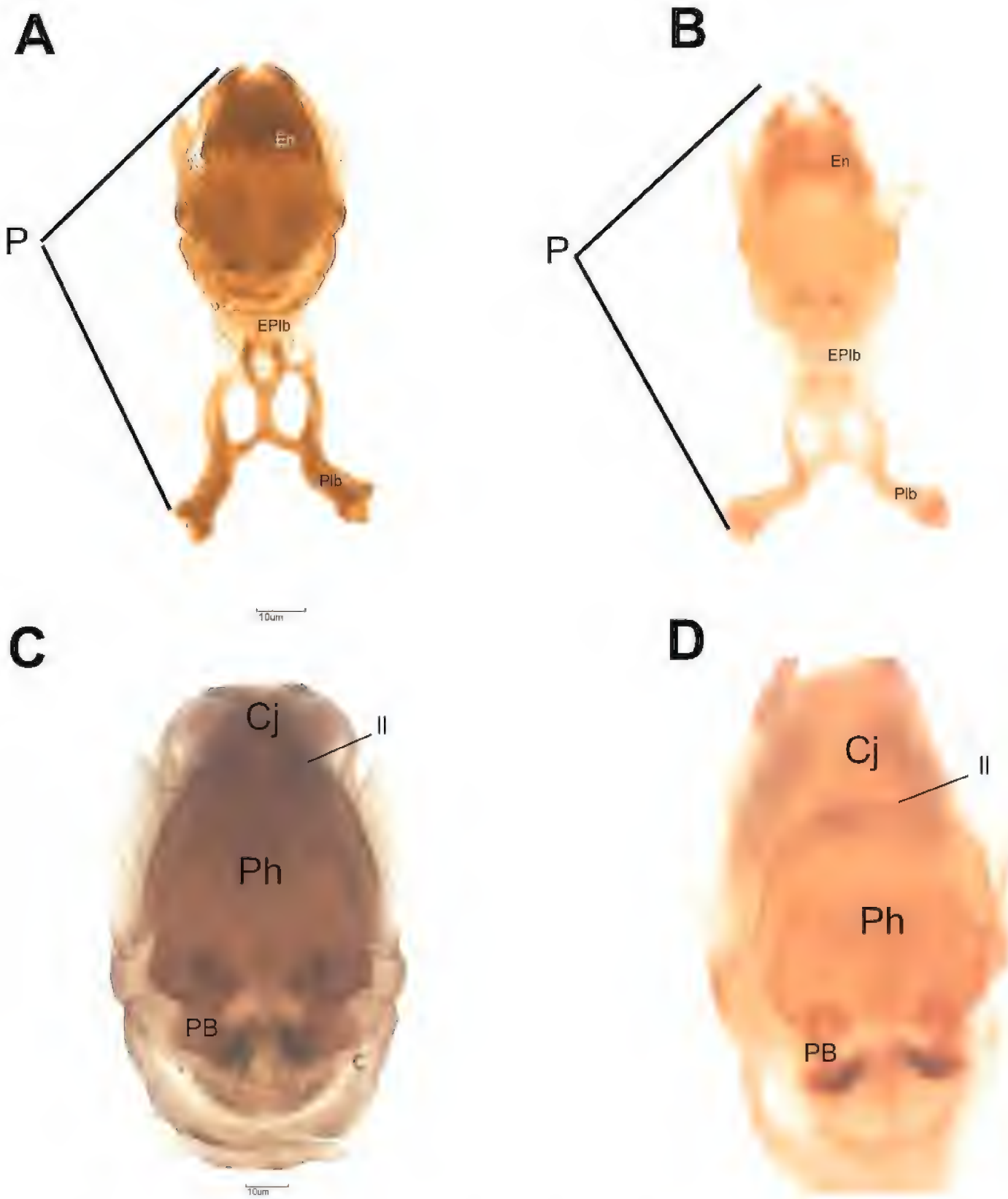

Figure 8. Phallus of *R. taquarussuensis* sp. n. **A** dorsal view **C** ventral view, *R. neglectus* **B** dorsal view **D** ventral view. Cj: conjunctive, En: endosome, EPlb: median extencion of basal plate, P: phallus, Plb: basal plate, PrG: gonopore process, PrPh: phallossoma process, Ph: phallosoma, PrCj: conjunctive process, ll: line limit.

The cuticle involving the veins of the hemelytron is light brown. The corium between the veins of the coriaceous region is dark brown, whereas that of the membrane is brown (Fig. 2A, C).

The prosternum contains the stridulatory sulcus, which moves along that segment in an antero-posterior direction, having a brown color in the background and black on

the sides. Two elongated tubercles limit the anterior half of the stridulatory sulcus. In the superior portion and in diagonal direction from the tubercles there are two black glabrous areas surrounded by a set of brown sensilla (Fig. 5A).

The mesosternum is limited anteriorly by the prosternum and posteriorly by the metasternum, both limits being brown. The central line dividing two dark brown elevations is also brown. Those two elevations are limited by two (1+ 1) black side glabrous areas diagonally placed. The central region of the posterior limit of the mesosternum has a half-moon shape. The metasternum is brown and resembles an isosceles triangle. Its anterior portion, i.e., its limit with the mesosternum, corresponds to the vertex of the triangle and is narrow, whereas its posterior portion, i.e., its limit with the first abdominal segment, corresponds to the basis of the triangle (Fig. 5C).

The three pairs of coxae are brown, except for the black glabrous areas. The trochanters of the anterior pair of legs are brown, but mixed with black glabrous areas. The middle and posterior pairs of trochanters are brown and have no glabrous areas. The three pairs of femora are black and the same color prevails in the three pairs of tibiae, except in the articulations with the femur and the spongy fossula, which are brown. The spongy fossulae are located in the first and second pairs of legs in the final portion of the tibia, alongside the articulations with the tarsi (Fig. 2A, B, C, D).

The abdomen of *R. taquarussuensis* sp. n. presents a brown color in the longitudinal central portion. On the sides of each segment there are (3+3) black glabrous areas, which are mixed with brown and black areas. The connexivum of the dorsal portion lies between the second and seventh segment. For each of those segments the anterior half is black and the posterior one is brown. The dorsal connexivum, also lying between the second and seventh segment, has a black color in 2/3 of the anterior portion, but that black color ends in an irregular way over the remaining 1/3, which is brown. Therefore, the black portion of the connexivum presents two edges moving towards the brown portion: one in the internal limit of the connexivum and the other in the middle portion. However, the connexivum of the second dorsal segment is black in the anterior half and brown in the posterior one, the limit between the portions having a diagonal shape. The seventh segment, on the other hand, is practically all black, except for a small brown strip located in the external posterior half. Type 1 sensilla, which prevail on the head, thorax and abdomen, have a brown color (Fig. 2B, D).

Male genitalia have the typical aspect of the genus *Rhodnius*. The median process of the pygophore (PrP) is short and triangular, but the base is broad and the sides are elongated with a thin edge. Parameres are hairy with a thin edge. From ventral view, the phallosome (Ph) has a broad plate whose superior region has a trapezoidal shape and occupies the middle region of the aedeagus. The support of the phallosome plate (PrPh) is broad. Conjunctival process I (PrcjI) is present and II (PrcjII) is absent. Endosomal process (En) is well-developed when seen from dorsal and ventral view (Figs 8A, C, 9B).

The dorsal side of the female external genitalia presents a concavity in the middle portion of the 10th segment. Seen from posterior view, the limits (1+1) of the 9th segment with gonocoxite VIII are curve, whereas the superior line limiting the 10thand 9thsegments is straight. In the central portion of the 10th segment of the ventral side of

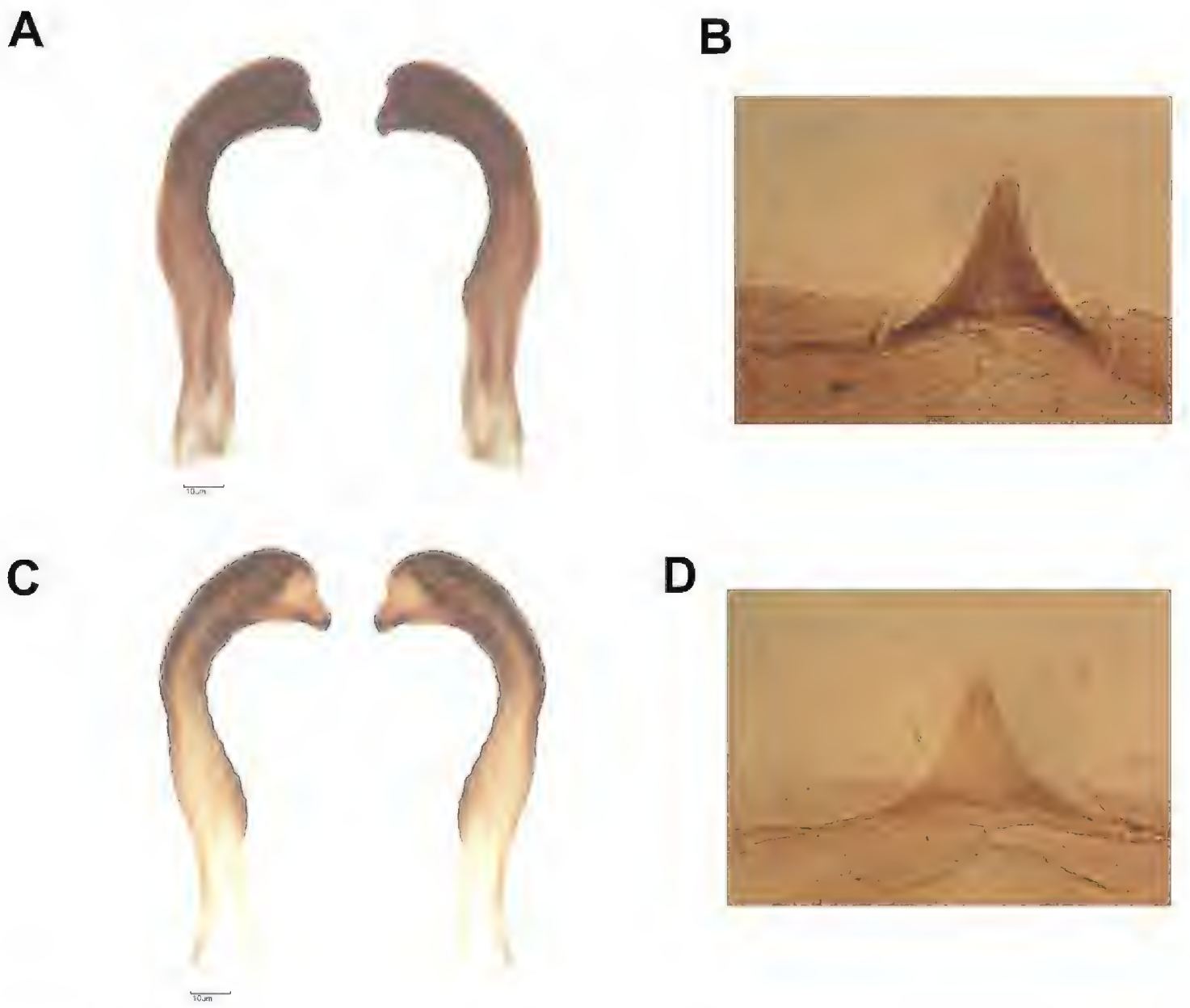

Figure 9. Parameres dorsal view of *R. taquarussuensis* sp. n. (**A**), Median process of the pygophore of *R. taquarussuensis* sp. n. (**B**) Parameres dorsal view of *R. neglectus* (**C**) Median process of the pygophore of *R. neglectus* (**D**).

the female external genitalia there is another concavity that can be noticed from dorsal view. The external limits (1+1) of the 9th segment of the female external genitalia are curve when seen from ventral view (Fig. 6A, C, E).

Egg shells of *R. taquarussuensis* sp. n. have a length of 1.72 mm and an opercular opening of 0.49 mm. They present lateral flattening, collar and exochorion cells, most with pentagonal or hexagonal shape (Fig. 7A, C).

Finally, although *R. taquarussuensis* sp. n. showed the same number of chromosomes as *R. neglectus* and all the tribe Rhodniini, i.e., 2n = 22 (Figure 11B), the constitutive heterochromatin pattern and the composition of the pairs of bases of DNA rich in AT and CG were completely different from *R. neglectus*, as the analysis of the nuclei of the initial prophases of *R. taquarussuensis* sp. n. has revealed a chromocenter consisting of sex chromosomes (arrow) and several heterochromatic blocks dispersed in the nucleus (Fig. 11A). The analysis of metaphase I of *R. taquarussuensis* sp. n. has demonstrated that this triatomine has heterochromatic blocks in both extremities of

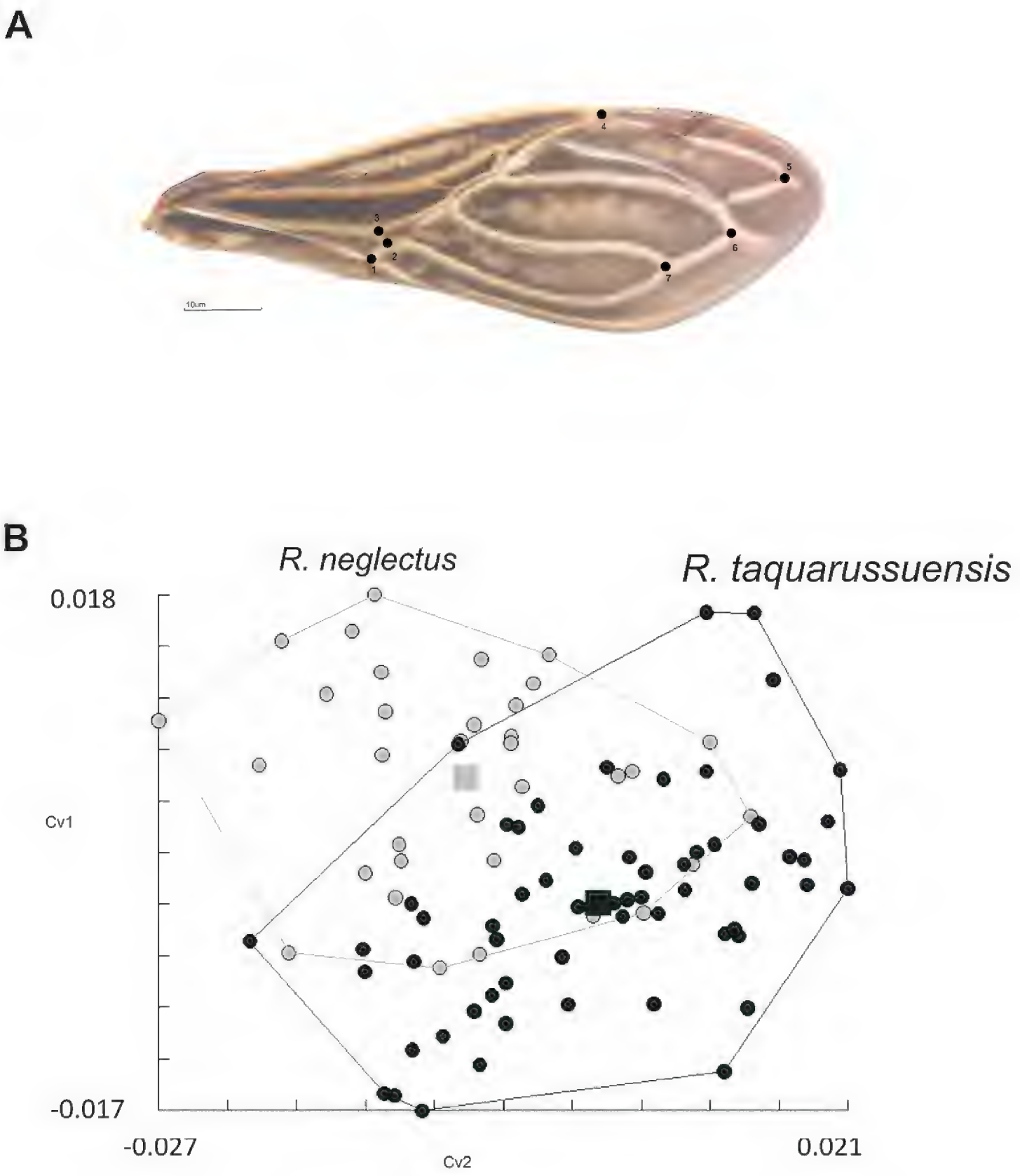

Figure 10. A Right wing of *R. taquarussuensis* sp. n. with the seven landmarks used in morphometric analysis. Following Bookstein (1990), all points correspond to type I landmarks (venation intersections) **B** Factorial maps in the plane of the two discriminant factors of wing shape variation (canonical variables 1 and 2, or CV1 and CV2) presenting the distribution of specimens of *R. taquarussuensis* sp. n. (Rta, black cicle) and *R. neglectus* (Rne, silver cicle).

practically all the autosomes and in the Y sex chromosome (Fig. 11B), unlike what has been recently stated for many populations of *R. neglectus* that do not present heterochromatin in autosomes (Alevi et al. 2015a). Furthermore, *R. taquarussuensis* sp. n. has the X sex chromosome rich in CG (Fig. 12C), the Y rich in AT (Fig. 12D) and various

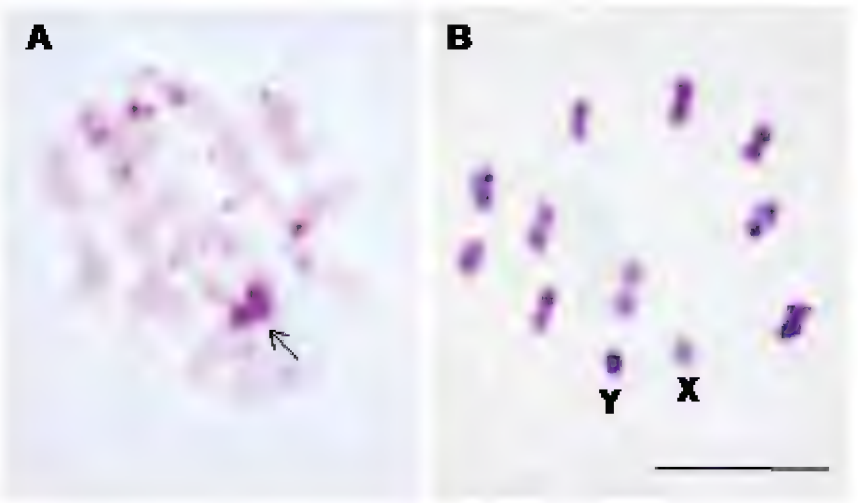

Figure 11. Constitutive heterochromatin pattern in *R. taquarussuensis*. **A** Initial prophases with a chromocenter heterochromatic consisting of sex chromosomes (arrow) and several heterochromatic blocks dispersed in the nucleus **B** Metaphase I with heterochromatic blocks in both extremities of practically all the autosomes and in the Y sex chromosome. X: X sex chromosome, Y: Y sex chromosome. Bar: 10 μm.

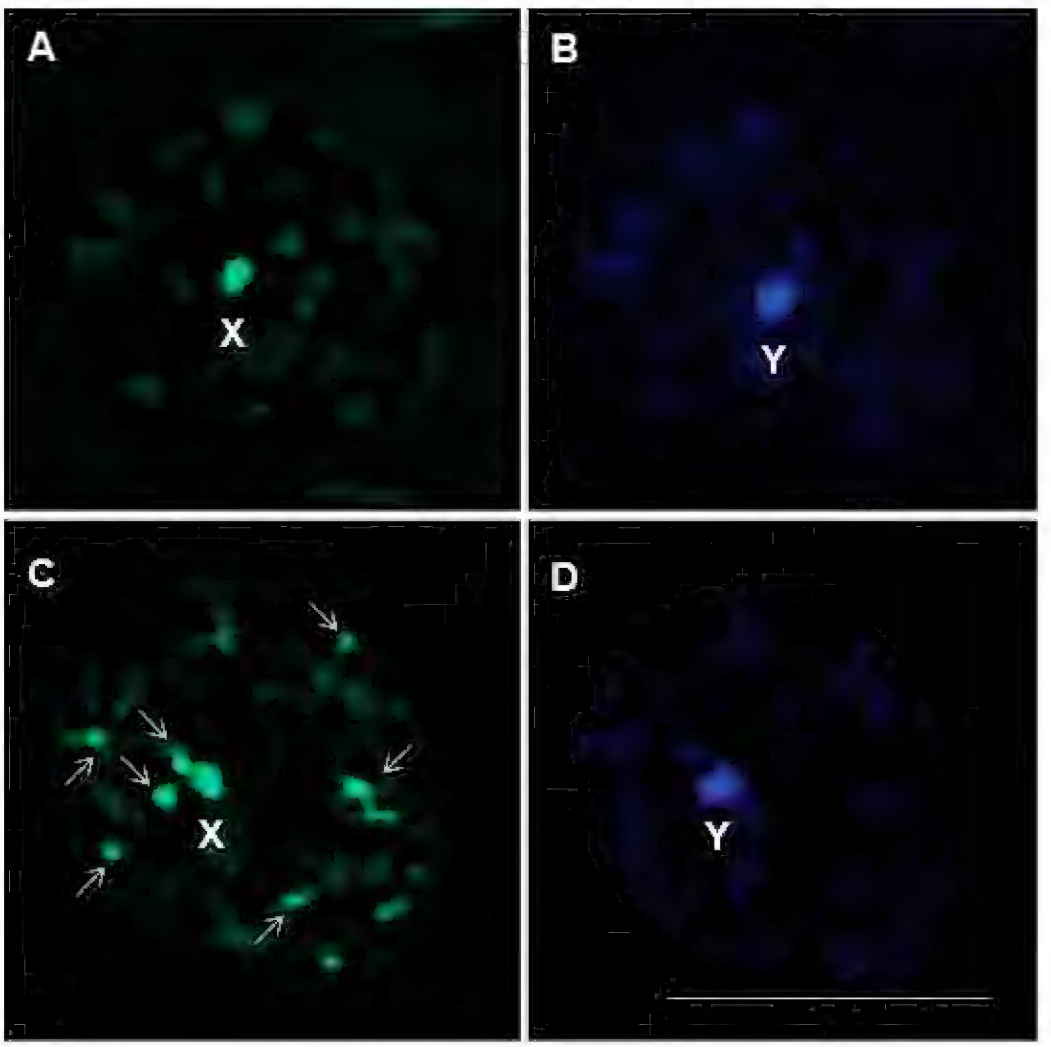

Figure 12. Composition of the pairs of bases of DNA rich in AT and CG in *R. neglectus* (**A, B**) and *R. taquarussuensis* (**C, D**). **A** X sex chromosome rich in CG **B** Y sex chromosome rich in AT **C** X sex chromosome and various blocks dispersed in the prophase nucleus (arrows) rich in CG **D** Y sex chromosome rich in AT. X: X sex chromosome, Y: Y sex chromosome. Bar: 10μm.

blocks rich in CG dispersed in the prophase nucleus (Fig. 12C), while *R. neglectus* only has the X sex chromosome rich in CG (Fig. 12A) and the Y rich in AT (Fig. 12B), which proves the genetic differences between the two *Rhodnius* species.

Discussion

The subfamily Triatominae include 18 genera comprising 152 species, 20 of which belong to the genus *Rhodnius* (Galvão 2014, Mendonça et al. 2016, Souza et al. 2016). The difficulties involved in the specific identification of *Rhodnius* have already been noted by Neiva and Pinto (1923), as well as by Rosa et al. (2012) and Souza et al. (2016). However, even though it is difficult to specify the distinctions among the species of that genus, in the last eight years four species were described: *R. zeledoni*, *R. montenegrensis*, *R. barretti*, and *R. marabaensis*. Therefore, *R. taquarussuensis* sp. n. is the 21st species of the genus and the 5th described in the last eight years. Its similarity with *R. neglectus* was noticed after the capture of the first female specimen, as the most evident macroscopic characters, such as size, general aspect and connexivum, showed no differences. As a result, it was decided to base its description on the differences from *R. neglectus*.

In addition to the macroscopic characters, *R. taquarussuensis* sp. n. and *R. neglectus* were considered "close to" because the OM study indicated similar characters between them, including: placement of black and brown spots on the dorsal and ventral connexivum, length of the four segments of the antenna, pronotum, antero lateral angles, urotergite I process, geometric morphometry of the hind wings, median process of the pygophore and morphological characters of the eggs.

Rhodnius taquarussuensis sp. n. was considered distinct from *R. neglectus* on account of the observation of color, eleven morphological characters, twelve morphometric characters and cytogenetic features (Tables 1, 2). All those differences are consistent with Lent and Wygodzinsky (1979), whose descriptive key to 11 species of *Rhodnius* lists 13 morphological characters for specific distinction: color, tibia, legs, pronotum, head, posterior lobe of the pronotum, anterolateral angles, median process of the pygophore, connexivum, scutellum, eyes and antennae.

Regarding the color, the distinction between *R. taquarussuensis* sp. n. and *R. neglectus* was based on the general aspect, segments of the antenna, hind wings and stridulatory sulcus. The general color of *R. taquarussuensis* sp. n. is brown, whereas *R. neglectus* is dark brown, almost black, or "brown dark", as referred to by Lent and Wygodzinsky (1979).

Out of the eleven morphological characters that distinguish *R. taquarussuensis* sp. n. from *R. neglectus*, three are located on the head: dorsal vertex, genae and triangular furrow of the first segment of the proboscis. The difference related to the vertex was one of the characters used by Souza et al. (2016) to distinguish *R. marabaensis* from *R. prolixus* and *R. robustus*. The differences between the genae for specific characterization are being reported for the first time in this description. The ventral triangular furrow was mentioned by Rosa et al. (1999) in their study of *T. rubrovaria* and it was mapped by Lent and Wygodzinsky (1979), but it was not named and it is being used for the first time as a distinctive character.

In what refers to the thorax, differences on the scutellum, prototHorax, mesothorax, and metathorax were noticed. The scutellum of *R. taquarussuensis* sp. n. and *R. neglectus* differs in the shape of the apex and also the position on which that apex reaches uroter-

gite I, since there is no significant difference in terms of length (Table 1). The taxonomic importance of the scutellum was tackled by Obara et al. (2007), who verified the differences of that character in eight species of *Triatoma*. Rosa et al. (2012) and Souza et al. (2016) also used it to describe *R. montenegrensis* and *R. marabaensis*, respectively.

The differentiation of seven genera of triatomines based on the shape of the prosternal stridulatory sulcus was carried out by Lent and Wygodzinsky (1979) and also by Souza et al. (2016) in the description of *R. marabaensis*. The description presented herein points out color and morphological differences between *R. taquarussuensis* sp. n. and *R. neglectus*. The mesothorax and metathorax, which have been used by Souza et al. (2016) to describe a new species, were found to be different in *R. taquarussuensis* sp. n. and *R. neglectus*.

Lent and Jurberg (1969) observed specific features in the characters of the male genitalia of 10 species of *Rhodnius* and since then that structure has been used to describe species of other genera of Triatominae, e.g., *Mepraia parapatrica* Frias, 2010, *P. mitarakaensis* Bérenger & Blanchet, 2007 and *T. jatai* Gonçalves et al., 2013. In the case of *R. taquarussuensis* sp. n. and *R. neglectus*, the difference in the male genitalia is the shape of the phallosome. With respect to the female external genitalia, differences between *R. taquarussuensis* sp. n. and *R. neglectus* could be observed on the dorsal, posterior and ventral sides, which differ from other 13 *Rhodnius* species, according to Rosa et al. (2014).

The eggs of *R. taquarussuensis* sp. n. and *R. neglectus* showed differences in the measurement of their length and opercular opening, the same as *R. montenegrensis* and *R. marabaensis* on the occasion of their description. As for the morphological characters, no differences were recorded. On the other hand, it should be noted that morphological differences were found by Barata (1981) in eggs of 10 *Rhodnius* species, by Santos et al. (2009) in three species, by Rosa et al. (2012) in the description of *R. montenegrensis* and by Santos et al. (2016) when describing *R. marabaensis*.

Dujardin et al. (1999) established the geometric morphometry of the hind wings as a distinctive character among Triatominae while studying the sexual dimorphism of *R. domesticus* and *T. infestans*. The technique proved valid, for instance, to indicate the distinction between *Mepraia spinolai* and *M. gajardoi*; *T. bahiensis* and *T. lenti*; *R. colombiensis*, *R. ecuadoriensis* and *R. pallescens*; five populations of *T. patagonica* (Campos et al. 2011, Díaz et al. 2014, Nattero et al. 2016). However, even though that technique has contributed to distinguish even very close species, it showed no significant results to distinguish *R. taquarussuensis* sp. n. from *R. neglectus*.

According to Justi and Galvão (2016) the group *R. prolixus* comprise the following species: *R. barretti*, *R. dalessandroi*, *R. domesticus*, *R. marabaensis*, *R.milesi*, *R.montenegrensis*, *R.nasutus*, *R. neglectus*, *R. neivai*, *R. prolixus* and *R. robustus*. Since *R.taquarussuensis* sp. n. is close to *R. neglectus* we suggest the inclusion of *R. taquarussuensis* sp. n. in the *R. prolixus* group and we present the main differences between the twelve species (Table 3).

Cytogenetic analyses of *R. taquarussuensis* sp. n. made it possible to describe the karyotype (2n = 22) and observe the constitutive heterochromatin pattern in the chro-

Table 3. Distinguishing characters among twelve species of the group *Rhodnius prolixus.*

Species	Distinctive characters	References
R. barretti	The third antennal segment appears to be relatively shorter. The scutellar process is narrowly pointed.	Abad-Franch et al. 2013
R. dalessandroi	Antenniferous tubercle slightly pilose and with triangular glabrous depression in the upper region. Semicircular spot on the posterior end of the neck.	Carcavallo and Barreto 1976
R. domesticus	Head comparatively long, distinctly longer that pronotum. Process of pygophore rectangular.	Lent and Wygodzinsky 1979
R. marabaensis	The second antennal segment is 10.3 times larger than the first. The scutellum is larger and includes two prominent internal lateral carinea.	Souza et al. 2016
R. milesi	The male genitalia presents a second process of the phallosoma. Divergent antennal tubercle with an apical denticle.	Valente et al. 2001
R. montenegrensis	Anterior wings with well-demarcated veins, notable the Sc by a yellow tonality. Abdomen presents yellow spots interposed with dark ones over the ventral abdomen lengthwise.	Rosa et al. 2012
R. nasutus	Overall color light reddish brown, trochantera not contrasting conspicuously with femora. Median process of pygophore wide at base.	Lent and Wygodzinsky 1979
R. neglectus	Overall color dark brown, trochantera very light colored. Median process of pygophore narrow at base.	Lent and Wygodzinsky 1979
R. neivai	Pronotum entirely dark brown or black, including the carine. Connexivum blackish, with very small reddish spots.	Lent and Wygodzinsky 1979
R. prolixus	Anteocular region slightly over three times as long as postocular. Distance between eyes dorsally larger than width of eyes in dorsal view.	Lent and Wygodzinsky 1979
R.robustus	Anteocular region about four times as long as postocular. Specimens distance between eyes dorsally smaller than, or equal to, width of eye in dorsal view.	Lent and Wygodzinsky 1979
R. taquarussuensis sp. n.	Head with a prominent brown vertex contrasting with the black sides. The phallosome (Ph) has a broad plate whose superior region has a trapezoidal shape and occupies the middle region of the aedeagus.	This work

*group *R. prolixus* according to Justi and Galvão 2016.

mosomes (extremities of most autosomes), which are rich in CG. All the species in the tribe Rhodniini have 22 chromosomes (Alevi et al. 2013, 2015b). On the other hand, out of the 14 species of the genus *Rhodnius* whose chromosomes have been studied in the literature, only four show heterochromatic blocks in the autosomes, namely *R. colombiensis*, *R. nasutus*, *R. pallescens* and *R. pictipes* (Dujardin et al. 2002). *R. neglectus*, which is a similar species for *R. taquarussuensis* sp. n., does not show heterochromatic blocks in the autosomes (Dujardin et al. 2002; Panzera et al. 2012; Alevi et al. 2015a).

Although the evolutionary process in triatomine is disruptive (Dujardin et al. 2009) and intraspecific chromosome variation has been described for *R. ecuadoriensis* (Pita et al. 2013), *R. pallescens* (Gómez-Palacio et al. 2008), *P. geniculatus* (Crossa-Pérez

et al. 2002), *T. dimidiata* (Panzera et al. 2006) *T. infestans* (Panzera et al. 2004, 2012) and *T. sordida* (Panzera et al. 1997), generally the distribution of species is associated with different countries [for exemple, *R. ecuadoriensis* from Peru and Ecuador (Pita et al. 2013) and *T. sordida* from Brazil and Argentina (Panzera et al. 1997)] or different regions [for example, *R. pallescens* from North and West regions from Colombia (Gómez-Palacio et al. 2008) and *T. infestans* from Andean group and Non-Andean group (Panzera et al. 2004, 2012)]. However, a population study was previously performed with *R. neglectus* (endemic species of Brazil) coming from different Brazilian states (Alevi et al. 2015a) and the authors pointed out that there is no intraspecific chromosome variation for this species. This fact and the morphological data described sustain the specific status of *R. taquarussuensis* sp. n., since the gain and loss of heterochromatin in the autosomes of *Rhodnius* are adaptive processes that can be linked to speciation processes, as recently noted for the group *pallescens* (Alevi et al. 2015c).

Authors' contributions

Conceived the study: JAR, HHGJ, JO and KCCA.Colected the bugs:HHGJ.

Prepared samples: JAR, JDN, JO, DBC and JDN. Analysed data: JAR, JO,VJM, RF,CSR and MTAO. Interpreted data: JAR, JO, KCCA, DBC. Wrote the manuscript: JAR, JO and KCCA.

All authors read and approved the final version of the manuscript.

Acknowledgents

Marcelo Ornaghi Orlandi, Mario Cilense e Sebastião Dametto from the Institute of Chemistry at São Paulo State University (UNESP) by the support and help regarding the use of the scanning electron microscope. Heloisa Pinotti, Eder dos Santos Souza, Lucas Abrantes da Silva, Tiago Belintani, Fábio Regis Garcia postgraduates of the Bioscience and Biotechnology Applied to Pharmacy program (FCF / UNESP) by the constant help in the maintenance of triatomine colonies. Nelson Papavero from the Zoological Museum of USP and Hélcio Reinaldo Gil-Santana of Diptera Laboratory, Oswaldo Cruz Institute for suggesting the scientific name of the new species, to Dennys Ortiz postgraduate of Animal Biology (UNICAMP) for the support with maps, to Davi Antonio da Rosa for the photos. The authors are also very grateful to Dr. Guanyang Zhang (Editor of Zookeys), Dr. Daniel A. Frias Lasserre (Laboratory of Entomology, Metropolitan University of Education Sciences, Santiago, Chile), Jean-Michel Bérenger (Laboratory Diagnostic Insect, Bouc-Bel-Air, France) and an anonymous reviewer for their valuable comments and suggestions. Financial Support: Scientific Support and Development Program of School of Pharmaceutical Sciences (UNESP);São Paulo Research Foundation (FAPESP) grant process number 2009/52236-2 and 2013/19764-0; Brazilian Federal Agency for the

Support and Evaluation of Graduate Education (CAPES), grant process number 23038-005285/2011-2012; Brazilian National Council for Scientific Technological Development (CNPq process number 142284/2015-7).

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